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(54) Title: STERILISING METHODS

(57) Abstract

Methods for sterilising pharmaceutical formulations and gels which are exposed to high energy radiation to destroy micro-organisms are characterised in that a free radical scavenger is incorporated in the formulation or gel to scavenge those free radicals which would degrade the pharmaceutical or the gel. The free radical scavenger and its products are substantially non-reactive with the gel and are substantially non-toxic. Particularly suitable free radicals scavengers are mannitol and ribitol.

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STERILISING METHODS

A first aspect of the present invention relates to a method for sterilising a pharmaceutical, in particular a pharmaceutical which is in aqueous solution.

The use of high energy radiations to destroy micro-organisms offers an alternative, and in some instances an attractive, means of sterilising pharmaceuticals compared with traditional techniques. In many instances it is cheaper, less energy consuming and provides better sterility assurance. Furthermore, it has none of the toxicity problems associated with gases (e.g. ethylene oxide) that are commonly used for sterilising purposes. However, radiation sterilisation of pharmaceuticals is often accompanied by chemical degradation, particularly in aqueous solutions, but also in other formulations such as gels, and this degradation must be minimised if the method is to be used successfully.

When dilute aqueous solutions are irradiated with high energy radiations (e.g. γ - or X-) the energy is completely absorbed by the water and produces highly reactive radicals ($H^{\cdot}$, $OH^{\cdot}$) and ions (e^{-}_{aq}).

$$H_2O \xrightarrow{\gamma-} e^{-}_{aq} + OH^{\cdot} + H^{\cdot}$$

The reactions of these species are responsible for degradation of solute present. When oxygen is present, e^{-}_{aq} and $H^{\cdot}$ are effectively removed:

$$e^{-}_{aq} + O_2 \rightarrow O_2^{-+H^+} HO_2^{\cdot} \quad (pk\ 4.8)$$
$$H^{\cdot} + O_2 \rightarrow HO_2^{\cdot}$$

HO_2 radicals are generally considered to be unreactive. Thus in oxygenated solutions, the $OH^{\bullet}$ radical (an oxidative species) causes chemical change, viz

$$RH + OH^{\bullet} \longrightarrow R^{\bullet} + H_2O \text{ (abstraction)}$$
$$\text{or } R'H + OH^{\bullet} \longrightarrow {}^{\bullet}R'H(OH) \text{ (addition)}$$

The radicals $R^{\bullet}$ and ${}^{\bullet}R'H(OH)$ then react with dissolved oxygen to form peroxy radicals $RO_2^{\bullet}$ or ${}^{\bullet}R'H(OH)O_2^{\bullet}$ and these subsequently disappear to form permanent degradative products. Thus, in many situations high energy-radiations cannot be used to sterilise aqueous solutions.

It is an object of the first aspect of the present invention to provide an improved method for sterilising a pharmaceutical.

According to a first aspect of the present invention, there is provided a method for sterilising a pharmaceutical formulation, wherein the pharmaceutical formulation is exposed to high energy radiation to destroy micro-organisms, characterised in that a free radical scavenger is incorporated in the formulation to scavenge those free radicals which could degrade the pharmaceutical, the said free radical scavenger and its products being substantially non-reactive with the pharmaceutical and being substantially non-toxic.

The free radical scavenger should be one which:

(i) is sufficiently soluble (in the formulation) to give good protection;

(ii) yields free radicals which will not degrade the pharmaceutical;

(iii) is non-toxic;

(iv) yields toxic products;

(v) does not cause protection to the bacteria (that are supposed to be killed).

Preferably the free radical scavenger is a polyhydric alcohol, for example mannitol or ribitol.

If the pharmaceutical formulation is an aqueous solution, the free radical scavenger is an additional solute in the aqueous solution and competes for the $OH^{\bullet}$ radicals. Thus the free radical scavenger must react readily with $OH^{\bullet}$ radicals, its own radiation degradation characteristics must be well known, it must be non-toxic, its products must be non-toxic and preferably easily metabolised, and for commercial reasons it should be inexpensive. These criteria are fully met in most circumstances by mannitol.

An embodiment of the first aspect of the present invention will now be described, by way of example.

Chloramphenicol is a pharmaceutical which is used in aqueous solution for eye-drops. Mannitol which is a polyhydroxyalcohol was introduced as a solute into aqueous solutions of chloramphenicol (hereinafter CP) and the resultant aqueous solutions were exposed to radiation at a variety of mannitol and CP concentrations. The mannitol reacts with OH radicals with a diffusion-controlled rate constant to yield almost entirely mannose and fructose (as assayed using GC and HPLC). The results are given in table 1 below.

Table 1

(a) The effects of γ-irradiation (25KGy) on CP (0.5% w/v) in the presence of increasing mannitol concentrations

% mannitol (w/v)	% destruction of CP	(in relation to mannitol) % mannose formed	% fructose formed
0.00	40.0	0.00	0.00
5.07	10.0	0.076	0.02
9.10	6.0	0.097	0.02
12.00	3.8	0.092	0.022
16.00	2.4	0.108	0.022

(b) The effects of γ-irradiation (25KGy) on CP at various concentrations with mannitol (0.27 $moldm^{-3}$)

CP Concentration %w/v	% Degradation of CP
0.07	1.25
0.17	2.00
0.26	3.80
0.30	4.50
0.41	5.10
0.50	10.00

Similar tests were then conducted with a range of polyhydric alcohols including mannitol and the results are given in Table 2:

Table 2

γ-irradiation of preoxygenated CP (0.5% w/v) with different polyhydric alcohols in borate buffer (1.5%), pH 7. Total dose received 25KGy. Dose rate 540y min^{-1}

polyhydric alcohols ($moldm^{-3}$)	% Remaining postirradiation HPLC Sample	HPLC CP	GC Sample	GC CP	pH	Colour	products formed $moldm^{-3}$			% calculated degradation of polyalcohols
-	-	50	-	60	7	intense yellow	-			-
Mannitol 0.27	100	97	100	98.5	7	almost colourless	Mannose $0.3x10^{-2}$	Fructose $0.2x10^{-2}$	3 or 4 Carbon compound	1.85
Sorbitol 0.3	99	88	100	108	7	"	Glucose $0.33x10^{-2}$	Fructose $0.1 x10^{-2}$	Arabinose 3,4 carbon $0.02x10^{-2}$	1.5
Galacticol 0.37	99	85	100	88	7	"	Galactose $0.75x10^{-2}$	Tagatose $0.09x10^{-2}$	3,4 carbon compound	3
Erythritol 0.41	99	94	98.8	92	7	"	Erythrose $0.45x10^{-2}$	-	-	1.1
Arabinitol 0.3	97.5	87	106	83	7	"	Arabinose $0.3 x10^{-2}$	-	3,4 carbon compound	1.0
Ribitol 0.33	107	83	98.5	94	7	"				
Glycerol 0.28	98.2	88	100	98	7	"				

The irradiation of the CP in the absence of a radical scavenger produces an intense yellow coloured solution indicating that substantial degradation had occurred. In the presence of a radical scavenger almost colourless solutions of the CP were obtained after irradiation thus indicating that no substantial degradation had occurred.

It can be seen from the results set out above that mannitol is particularly effective in protecting CP against degradation but other polyhydric alcohols also produce good results.

A second aspect of the invention relates to the sterilisation of alginate gels. Such gels cannot currently be sterilised by irradiation or by heating.

For example, gels prepared from 1%, 2% and 3%

alginate all lose water following irradiation to a final dose of 25KGy. The gels also shrink in size, become brittle and are easily broken on bending. Moreover, they lose their ability to take up saline and water and cannot be handled or manipulated.

It is an object of the second aspect of the invention to obviate or mitigate this problem.

According to a second aspect of the present invention there is provided a method for sterilising a gel, preferably an alginate gel, wherein the gel is exposed to high energy radiation to destroy micro-organisms, characterised in that a free radical scavenger is incorporated in the gel to scavenge those free radicals which could degrade the gel, the said free radical scavenger and its products being substantially non-reactive with the gel and being substantially non-toxic.

The free radical scavenger used in the second aspect of the invention should meet the requirements set out for those used in the first aspect of the invention. Preferably, the free radical scavenger for the second aspect of the invention is mannitol or ribitol.

The second aspect of the invention is illustrated by the following description.

For example an unirradiated 2% alginate gel containing 22% mannitol takes up 16% of its own weight of saline. After irradiation to a dose of 25KGy the same gel will take up 7% saline. When mannitol is absent the unirradiated gel takes up 14% saline and after irradiation (25KGy) takes up 1% of saline.

A further example concerns gel bending. A 2% alginate gel containing 18% mannitol can be bent through an angle of 180° i.e. it is fully flexible.

The same gel without mannitol is also fully flexible. After irradiation (25KGy) the mannitol containing gel can still be bent through an angle of 80°. In marked contrast the gel irradiated without mannitol breaks when bent through an angle of 10°. Such a gel is not usable.

When a 2% alginate gel containing 18% mannitol is prepared with an open nylon mesh incorporated into it. The gel, even after a dose of 25KGy can be bent through 180° (i.e. is fully flexible) and moreover is easily manipulated.

CLAIMS

1. A method for sterilising a pharmaceutical formulation wherein the pharmaceutical formulation is exposed to high energy radiation to destroy micro-organisms, characterised in that a free radical scavenger is incorporated in the formulation to scavenge those radicals which could degrade the pharmaceutical, the said free radical scavenger and its products being substantially non-reactive with the pharmaceutical and being substantially non-toxic.

2. A method as claimed in Claim 1 wherein the pharmaceutical is in aqueous solution.

3. A method as claimed in Claim 1 or 2 wherein the free radical scavenger is a polyhydric alcohol.

4. A method as claimed in Claim 3 wherein the polyhydric alcohol is mannitol or ribitol.

5. A method as claimed in any one of Claims 1 to 4 wherein the pharmaceutical is chloramphenicol.

6. A method as claimed in any one of the preceding claims wherein the pharmaceutical formulation to be sterilised comprises upto 20% by weight of the free radical scavenger.

7. A method as claimed in any one of the preceding claims wherein the pharmaceutical formulation to be sterilised comprises up to 10% by weight of the free radical scavenger.

8. A method as claimed in any one of the preceding claims wherein the pharmaceutical formulation comprises up to 1% by weight of the pharmaceutical.

9. A method for sterilising a pharmaceutical formulation substantially as hereinbefore described.

10. A method for sterilising a gel, wherein the gel is exposed to high energy radiation to destroy microorganisms characterised in that the free radical

scavenger is incorporated in the gel to scavenge those free radicals which could degrade the gel, the said free radical scavenger and its products being non-reactive with the gel and being substantially non-toxic.

11. A method as claimed in Claim 10 wherein the gel is an alginate gel.

12. A method as claimed in Claim 10 and 11 wherein the free radical scavenger is a polyhydric alcohol.

13. A method as claimed in Claim 12 wherein the polyhydric alcohol is mannitol or ribitol.

14. A method as claimed in any one of Claims 10 to 13 wherein the gel is an alginate gel.

15. A method as claimed in any one of Claims 10 to 14 wherein the gel to be sterilised comprises up to 25% of the free radical scavenger.

16. A method for sterilising a gel substantially as hereinbefore described.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/00839

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 L 2/08

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]:	A 61 L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Microbiology Abstracts, section A, vol. 9, no. 10, October 1973 Information Retrieval Ltd. (London, GB) H. Affolter et al.: "The antimicrobial treatment of medicaments by gamma-rays" see page 94, abstract 9A6126 & Pharm. Acta Helvetica, 48(10), 525-40, 1973 --	1,3
X	Chemical Abstracts, vol. 73, no. 13, 28 September 1970, (Columbus, Ohio, US), G. Hangay et al.: "Radiosterilization of an ophtalmic ointment containing hydrocortisone and chloramphenicol" II. Ointment base constituents" see page 226, abstract 69804q & Acta Pharm. Hung. 1970, 40(2), 75-80 ----------	1,3,5,9

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
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